1-Methyl-3-methoxy-16β-ethylthioestra-1,3,5(10)-trien-17β-ol Acetate (XV)——Conversion of XIV to the corresponding ethylthio derivative was carried out according to the procedure described in the earlier paper.[1] The product was reduced with $LiAlH_4$ and, without further purification, acetylated by treating with pyridine and Ac_2O to give 2.25 g. of XV. Recrystallization from MeOH yielded colorless plates, m.p. 86.5~87°, $[\alpha]_D^{24}$ +149.9±2° (c=1.030). UV λ_{max} mμ (ε): 226.5 (9650), 279 (1630), 286 (1680). IR ν_{max}^{Nujol} cm^{-1}: 1736, 1237 (O-Ac). *Anal.* Calcd. for $C_{24}H_{34}O_3S$: C, 71.61; H, 8.51; S, 7.95. Found: C, 71.49; H, 8.54; S, 7.68.

Summary

Some estratrienones having a sulfur atom at C-16 were prepared by substitution of 16α-bromo-17-ketosteroids with sulfur nucleophiles.

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UDC 581.19 : 582.572.2 : 547.92

91. Ken'ichi Takeda, Ariyoshi Shimaoka, Mitsutaka Iwasaki, and Hitoshi Minato: Studies on the Steroidal Components of Domestic Plants. XLVIII.*1 Components of *Chionographis japonica* Maxim. (1).

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Chionographis japonica Maxim. is a pernnial herb belonging to Liliaceae and its components have not hitherto been investigated. In this paper, we will report on the steroidal components of this plant. The dried whole plant (1.2 kg.) was extracted with methanol, and the methanol extract was saponified with 5% sulfuric acid in methanol and extracted with benzene. As the thin-layer chromatogram of this extract showed many spots, it was chromatographed on alumina to give the ten-compounds (A~K: except I), shown in Table I.

Table I. Fractions obtained from the Benzene Extract

		Rf value of thin-layer chromatogram[a]	Color test[b]	Yield (mg.)
Compound	A (diosgenin I)	0.93	yellow	560
	B (bethogenin IIa)	0.86	〃	74
	C (β-sitosterol III)	0.80	blue-violet	26
	D (pennogenin IV)	0.75	yellow	130
	E (unknown)	0.73	reddish-purple	50
	F (kryptogenin Va)	0.69	orange-yellow	32
	G (chiogralactone)	0.65	reddish-purple	62
	H (unknown)	0.41	red	85
	J (〃)	0.20	〃	41
	K (〃)	0.15	blue-violet	385

a) solvent system: $CHCl_3$-acetone-acetic acid=27:2:1
b) color test: 5% cinnamic aldehyde in ethanol-sulfuric acid

*1 Part XLVII. T. Okanishi, A. Akahori, F. Yasuda: This Bulletin, 13, 545 (1965).
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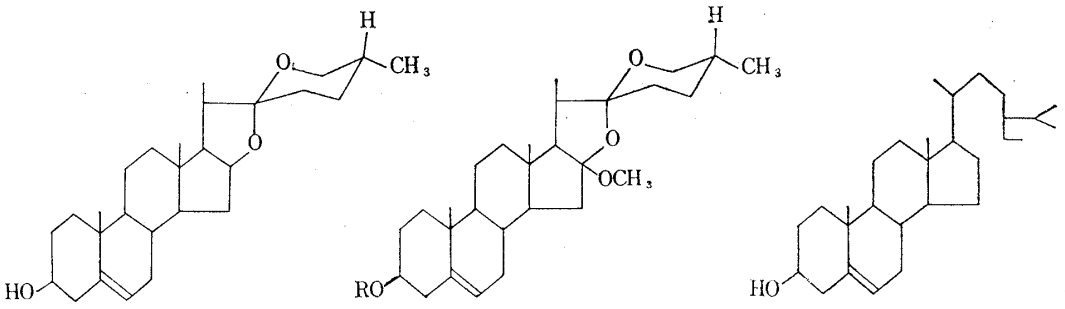
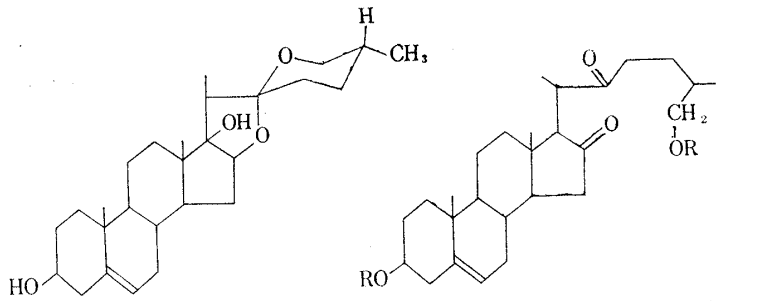
Compound A (I) was recrystallized to give colorless needles, m.p. 202~203°, $[\alpha]_D$ −127°, which was identical with diosgenin by mixed melting point determination and comparisons of infrared spectra and $[\alpha]_D$ values. Compound B (IIa) was recrystallized from methanol to give colorless needles, m.p. 160~165°, and from methanol containing 2% potassium hydroxide[1] to give colorless needles, m.p. 192~194°, $[\alpha]_D$ −96.0°. The acetate (IIb) was obtained as colorless prisms, m.p. 211~212°, $[\alpha]_D$ −101.9°. The physical constants of IIa and IIb are consistent with those of bethogenin. Moreover, when the methanol extract of the plant was saponified with 5% sulphuric acid in ethanol, compound B was not obtained. Compound (IIa), therefore, was assumed to be bethogenin. Compound C (III), colorless plates, m.p. 134~135°, was identical with β-sitosterol by mixed melting point and infrared spectra. Compound D (IV), colorless needles, m.p. 242~243°, $[\alpha]_D$ −104.1° was established to be identical with pennogenin by mixed melting point and infrared spectra. Compound F (Va) was acetylated with acetic anhydride-pyridine, and its acetate (Vb) was recrystallized from methanol to give colorless plates, m.p. 151~152°, $[\alpha]_D$ −160°, which was identical with kryptogenin diacetate by mixed melting point and infrared spectra.



I IIa, b III



IV Va, b a : R=H b : R=$COCH_3$

Compound G was obtained as colorless prisms, m.p. 232~234°, $[\alpha]_D$ −113.4° and had the empirical formula, $C_{23}H_{34}O_4$. This compound is not a steroidal sapogenin but shows a ketonic group (1712 cm^{-1}) and a δ-lactonic function (1733 cm^{-1}) in the infrared spectrum. Acetylation with acetic anhydride in pyridine at room temperature afforded a monoacetate, m.p. 228~230°, the infrared spectrum of which no longer showed an absorption band corresponding to the hydroxyl group. Moreover, compound G gave a diketone, m.p. 198~200°, by oxidation with chromium trioxide. From these results, compound G is an unknown compound having a hydroxyl group, a ketonic group and

1) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, C. H. Ruof: J. Am. Chem. Soc., **69**, 2167 (1947).

a δ-lactonic function, and was named "chiogralactone." Confirmation of its structure is now under investigation in our laboratory.

Compound E was obtained as colorless plates, m.p. 128~130°, $[\alpha]_D$ −35.1°, and had the empirical formula, $C_{29}H_{46}O_5$. Compound H could not be obtained in the pure state. Compound J, colorless prisms, m.p. 125.5~128°/206~210.5°, $[\alpha]_D$ −6.8°, had the empirical formula, $C_{27}H_{46}O_5$ and afforded the tetracetate, m.p. 210~211.5° The remaining one oxygen is present in a ketonic group (1708 cm^{-1}). Compound K, colorless plates, m.p. 240~243.5°, $[\alpha]_D$ +16.1°, corresponds to the empirical formula $C_{27}H_{48}O_5$, and afforded the oily pentacetate and the pentabenzoate, m.p. 150~152°.

As compound E, H, J, and K all lack the characteristic bands of the E- and F-rings of the steroidal sapogenin in the infrared spectra, they are not steroidal sapogenins. In spite of the fact that steroidal components of hitherto investigated plants belonging to Liliaceae are almost the steroidal sapogenins, it is noteworthy that compounds other than steroidal sapogenins are present as the major components in *Chionographis japonica* MAXIM.

Experimental*3

Isolation of the Sapogenins from the Whole Plant——The dried and sliced whole plant (1.2 kg.) was extracted with 80% hot MeOH giving a deep brown syrup, which was extracted with benzene. The benzene-insoluble residue was extracted with BuOH (5 L.×2) to give a deep brown syrup (110 g.). The syrup was dissolved in a solution of conc. H_2SO_4 (200 g.) in 80% MeOH (4 L.), refluxed for 6 hr., poured into a great amount of H_2O, and extracted with benzene leaving a deep brown syrup (7 g.). The residue was dissolved in benzene (300 ml.) and chromatographed on Al_2O_3 (300 g.; see Table II).

TABLE II. Alumina Chromatogram of the Benzene Extract

Fraction No.	Solvent	Rf value	Yield (g.)
1~ 9	benzene	——	1.20
10~14	benzene-$CHCl_3$ (9:1)	0.93	1.04
15~17	〃 (4:1)	0.86, 0.80	0.45
18~19	〃 (1:1)	0.80, 0.75	0.15
20~25	$CHCl_3$	0.75, 0.73	0.29
26~34	〃	0.73, 0.69, 0.65	1.40
35~39	$CHCl_3$-MeOH (9:1)	0.41, 0.20, 0.15	2.50

Compound A (Diosgenin, I)——Fractions (10~14) were crystallized from MeOH giving compound A (I, 560 mg.) as colorless needles, m.p. 202~203°, $[\alpha]_D^{23}$ −127°(±2°) (c=1.02), Rf value 0.93. Compound A and its acetate are identical with diosgenin (I) and its acetate by mixed melting points, IR spectra and $[\alpha]_D$ values.

Compound B (Bethogenin, IIa)——Fractions (15~17) were rechromatographed on Al_2O_3, and recrystallized from MeOH giving compound B, bethogenin (IIa, 74 mg.) as colorless needles, m.p. 160~165°, and from MeOH containing 2% KOH[1] as colorless needles, m.p. 192~194°, $[\alpha]_D^{24}$ −96.0°(±2°) (c=1.064), Rf value 0.86. *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97; OCH_3, 6.98. Found: C, 75.56; H, 10.04; OCH_3, 6.44. Acetate (IIb), colorless prisms, m.p. 211~212°, $[\alpha]_D^{25}$ −101.9°(±4°) (c=0.591). *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found: C, 74.16; H, 9.52.

Compound C (β-Sitosterol, III)——Fractions (18~19) were rechromatographed on Al_2O_3 and recrystallized from MeOH giving compound C (III, 26 mg.) as colorless plates, m.p. 134~135°, Rf value 0.80. Compound C is identical with β-sitosterol (III) by mixed melting point and IR spectra.

Compound D (Pennogenin, IV)——Fractions (20~25) were recrystallized from MeOH giving compound D (IV, 130 mg.) as colorless needles, m.p. 242~243°, $[\alpha]_D^{25}$ −104.1°(±2°) (c=1.081), Rf value 0.75. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.81. Found: C, 75.03; H, 10.03. Compound D is identical with pennogenin (IV) by mixed melting point, IR spectrum and $[\alpha]_D$ value.

Isolation of Compound E, F (Va) and G——Fractions (26~34) were dissolved in benzene (100 ml.) and rechromatographed on Al_2O_3 (40 g.) as shown in Table III.

*3 All meltiog points were taken on the Kofler block and corrected. Unless otherwise specified, IR spectra and $[\alpha]_D$ values were taken in $CHCl_3$. Thin-layer chromatography was carried out with "Merck," Kieselgel G and $CHCl_3$-acetone-AcOH (27:2:1).

Table III. Alumina Chromatogram of Fractions (26～34)

Fraction No.	Solvent	Rf value	Yield (mg.)
1～ 2	benzene	0.93, 0.75, 0.73	64
3～ 4	benzene-$CHCl_3$ (95:5)	0.93, 0.75, 0.73	75
5～ 6	〃 (9:1)	0.75, 0.73, 0.65	295
7～ 8	〃 (4:1)	0.73, 0.65	184
9～14	〃 (1:1)	0.65, 0.69	434
15～16	$CHCl_3$	0.65, 0.69	117
17～18	〃	0.69	60
19～21	$CHCl_3$, $CHCl_3$-MeOH (9:1)	0.69	130

Fractions (5～8) gave compound E by rechromatography on Al_2O_3, preparative thin-layer chromatography and recrystallization from acetone-pentane as colorless plates, m.p. 128～130° (50 mg.), $[\alpha]_D^{24}$ −35.1° (±3°) (c=0.696), Rf value 0.73, ν_{max} 1710 cm^{-1}. *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found : C, 73.36; H, 9.90. Acetate, colorless prisms, m.p. 168～170°.

Fractions (17～21) were acetylated with $(CH_3CO)_2O$-pyridine to give an oily acetate (100 mg.), which gave compound F diacetate (Vb) by rechromatography on Al_2O_3, preparative thin-layer chromatography and recrystallization from MeOH as colorless plates, m.p. 151～152° (32 mg.), $[\alpha]_D^{24}$ −160° (±6°) (c=0.219). *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found : C, 72.25; H, 9.04. Compound F diacetate is identical with kryptogenin diacetate (Vb) by mixed melting point, IR spectrum and $[\alpha]_D$ value.

Fractions (9～16) gave compound G (chiogralactone) by rechromatography on Al_2O_3, preparative thin-layer chromatography and recrystallization from acetone-ether as colorless prisms, m.p. 232～234° (62 mg.), $[\alpha]_D^{24}$ −113.4° (±6°) (c=0.277), Rf value 0.65, ν_{max} 1733 and 1712 cm^{-1}. *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found : C, 73.65; H, 9.16. Acetate, colorless plates (from ether-acetone), m.p. 228～230°. *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found : C, 71.83; H, 8.75.

Oxidation of Chiogralactone (Compound G)——Chiogralactone (10 mg.) was oxidized with Jones' reagent to give diketone (6 mg.), colorless plates, m.p. 212～214° (from acetone-hexane). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found : C, 73.88; H, 8.86.

Reduction of Chiogralactone (Compound G) with Sodium Borohydride——Chiogralactone (10 mg.) was treated with $NaBH_4$ (5 mg.) in MeOH (1 ml.) to give dihydroxy compound (9 mg.), colorless prisms, m.p. 280～283° (from MeOH-$CHCl_3$). *Anal.* Calcd. for $C_{23}H_{36}O_4$:C, 73.36; H, 9.64. Found: C, 73.41; H, 9.67.

Isolation of Compound H, J and K——Fractions (35～39 in Table II) were recrystallized from acetone to give compound K (385 mg.) as colorless plates, m.p. 240～243.5°, $[\alpha]_D^{24}$ +16.1° (±4°) (c=0.490, MeOH), Rf value 0.15. *Anal.* Calcd. for $C_{27}H_{48}O_5 \cdot \frac{3}{4}H_2O$: C, 69.52; H, 10.73; H_2O, 2.90; mol. wt., 466.2 (452.6). Found : C, 69.28; H, 10.74; H_2O, 2.98; mol. wt., 438. [Diacetate colorless needles, m.p. 248～249° (from acetone). *Anal.* Calcd. for $C_{31}H_{52}O_7$: C, 69.37; H, 9.77; O, 20.87; mol. wt., 536.7. Found : C, 69.40; H, 9.79; O, 20.86; mol. wt., 561. Monoacetate, colorless plates, m.p. 125～126° (decomp.) (from $CHCl_3$-petr. ether). Pentabenzoate, colorless prisms, m.p. 150～152° (from MeOH). *Anal.* Calcd. for $C_{62}H_{68}O_{10}$: C, 76.52; H, 7.04. Found : C, 76.08; H, 7.08.].

The residue (2.1 g.) from recrystallization of compound K was dissolved in a solution of Girard's reagent T (4.2 g.) in EtOH (200 ml.) and AcOH (20 ml.) and refluxed for 3 hr. to give a mixture of carbonyl compounds (360 mg.), which was chromatographed on Al_2O_3 (10 g.). Elution with $CHCl_3$ and $CHCl_3$-MeOH (98:2) afforded a viscous oil (190 mg.), which was rechromatographed on Al_2O_3 to give compound H, a viscous oil (85 mg.), Rf value 0.41, ν_{max} 1715 cm^{-1}. Further elution with $CHCl_3$-MeOH (95:5～80:20) afforded a viscous oil (109 mg.), which was crystallized from benzene to give compound J (41 mg.) as colorless prisms, m.p. 125.5～128°/206～210.5°, $[\alpha]_D^{24}$ −6.8° (±6°) (c=0.311, MeOH), Rf value 0.20, ν_{max} 1708 cm^{-1}. *Anal.* Calcd. for $C_{27}H_{46}O_5 \cdot \frac{1}{2}H_2O$: C, 70.55; H, 10.30. Found : C, 70.28; H, 10.14. Acetate, colorless plates, m.p. 210～211.5° (from acetone-petr. ether). *Anal.* Calcd. for $C_{35}H_{54}O_9$: C, 67.93; H, 8.80. Found : C, 67.86; H, 8.84.

Summary

The steroidal constituents of *Chionographis japonica* Maxim. were investigated, and four steroidal sapogenins, diosgenin (I), bethogenin (IIa), pennogenin (III) and kryptogenin (Va), were isolated. Moreover, chiogralactone and four unknown compounds were obtained as compounds other than steroidal sapogenins.

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